PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

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PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY
(PCT Rule 43*bis*.1)

Date of mailing
(day:month:year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day:month:year)*	Priority date *(day/month/year)*
PCT/US2005/001993	21.01.2005	23.01.2004

International Patent Classification (IPC) or both national classification and IPC
INV. G01N33/573

Applicant
SANOFI PASTEUR, INC.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:	Date of completion of this opinion	Authorized Officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	see form PCT/ISA/210	Weijland, Albert Telephone No. +49 89 2399-7490

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2005/001993

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed

   ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1 (b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

      ☐ a sequence listing

      ☐ table(s) related to the sequence listing

   b. format of material:

      ☐ on paper

      ☐ in electronic form

   c. time of filing/furnishing:

      ☐ contained in the international application as filed.

      ☐ filed together with the international application in electronic form.

      ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	
	No:	Claims	1-8
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-8
Industrial applicability (IA)	Yes:	Claims	1-8
	No:	Claims	

2. Citations and explanations

see separate sheet

The following documents (D) are referred to in this opinion; the numbering will be adhered to the rest of the procedure:

D1: US2003/0211548
D2: WO2005/049800
D3: APOPTOSIS 2003, VOL. 8, PAGES 563-571

1. The subject matter of claims 1-8 is anticipated by D1 to D3 and is therefore not novel (Article 33(2) PCT).

   D1 (abstract; example 5) describes a non-radioactive assay to monitor target-cell killing activities mediated by CTL, since the apoptosis pathway activation and caspase activity are a measure of such activity. The cytotoxicity is measured by the cleavage of a cell permeable caspase substrate ("detecting reagent" according to claim 1) using flow cytometry, after incubation of the fluorescently labelled cells ("target cells" according to claim 1) with the cytotoxic effector cells.

   D3 (abstract; page 567, right column, third paragraph) describes a flow-cytometric based assay for CTL-mediated cytotoxicity based on the binding of antibody to activated caspase-3 in target cells. This assay is more sensitive than the Cr-release assay. The target cells are stained with red fluorescent dye PKH-26. After co-incubation with CTL, the mixture is fixed with paraformaldehyde ("fixing and permeabilizing" according to claim 1), permeabilized and stained with a FITC-conjugated anti-caspase 3 antibody ("detecting reagent" according to claim 1). The cells are analysed by two colour flow cytometry.